Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

PRELIMINARY REPORT OF EXPERIMENTS IN THE VITAL STAINING OF TUBERCLES.*

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS. IV.

LYDIA M. DEWITT, B.S., M.D.

(From the Otho S. A. Sprague Memorial Institute and the Pathological Laboratory of the University of Chicago.)

In the rapid advance in our knowledge of scientific chemotherapy during the last few years, the various benzidene dyes have held no small or unimportant place. Since Ehrlich and Shiga[1] in 1904 published the results of their investigations on the trypanocidal action of trypan red, a new impetus has been given to the study of vital stains and especially to their use as therapeutic agents.

In 1906 Nicolle and Mesnil[2] reported an exhaustive and systematic study of dyes of the benzidene series (especially those resembling or closely related in their structure to trypan red) with regard to their vital staining and their trypanocidal power, the two being, they think, closely related. In the same year, Bouffard[3] published a communication along the same line, and numerous reports have come from Ehrlich and his workers, each adding something to our knowledge of vital stains which can be used therapeutically.

Goldmann[4] reported in 1909 an exhaustive investigation of the effect on the tissues of the normal body of a group of dyes, including the trypan red and the trypan blue already mentioned by Ehrlich and his workers, and comparing with them two other dyes which act in a similar way—isamine blue and pyrrhol blue. All these dyes have the common property of vitally staining granules in certain interstitial cells very deeply and permanently and thus giving a general and lasting stain to the body. After Goldmann's introduction of these dyes, a considerable number of workers

---

*Received for publication, November 29, 1912.

[1] *Berl. klin. Wchnschr.*, 1904, 41, p. 329; *ibid.*, 1907, 44, pp. 233, 280, 310, 341.

[2] *Ann. de l'Inst. Past.*, 1906, 20, p. 417.

[3] *Ibid.*, p. 539.

[4] *Beitr. z. klin. Chir.*, 1909, 64, p. 192.

investigated by means of these stains various physiologic and pathologic processes in the animal body, and during 1912 several communications from Goldmann have appeared, as well as two from Schulemann,[1] one from Lewis,[2] one from Bowman, Winternitz, and Evans,[3] and one from MacCurdy and Evans as well as several others which do not so especially have to do with my own work and which may therefore remain unmentioned.

Among all these workers, it is natural that some should be interested in the relation of these dyes to tuberculosis. Goldmann[4] himself in several of his later papers reports the results of his investigations on tuberculosis in mice, especially in regard to the origin and histogenesis of the tubercle. Bowman, Winternitz, and Evans, using rabbits, endeavored by means of the trypan blue dye to ascertain the histogenesis of the liver tubercle. Lewis showed that trypan red and isamine blue injected, intravenously or intraperitoneally, into tuberculous rabbits penetrated the pulmonary tubercles in the few cases which he reports.

Beyond suggesting the possibility that these results may some time be of use in the treatment of tuberculosis, none of these workers has taken up the chemotherapeutic value of vital stains in tuberculosis. Very recently, however, a series of papers has appeared dealing with the so-called "Finkler's Heilverfahren," the several papers being by Gräfin v. Linden,[5] Meissen,[6] Strauss,[7] and Selter.[8] The method uses either methylene blue (chlorid or iodid), or copper compounds, or both together. More or less favorable results were reported in the few experimental animals tested with the methylene blue treatment and also in a number of human tuberculous patients.

My own work began in February, 1912, and has been carried on with the definite purpose of testing dyes which can be used *intra vitam* to find if any have a specific action on the tubercle. The work is by no means finished, but certain results already obtained seem worth reporting at this time.

[1] *Berl. klin. Wchnschr.*, 1912, 49, p. 497; *Beitr. z. Vitalfärbung, Arch. f. mik. Anat.*, 1912, 79, p. 223; *Ztschr. f. exp. Pathol.*, 1912, 11, p. 307.

[2] *Arch. of Int. Med.*, 1912, 10, p. 68.

[3] *Centralbl. f. Bakt.*, I, Orig., 1912, 65, p. 403.

[4] *Verhandl. d. path. Gesellsch.*, 1910, 14, p. 138; *Beitr. z. klin. Chir.*, 1912, 78, p. 1; *Lancet*, 1912, 182, p. 1183; *Berl. klin. Wchnschr.*, 1912, 49, p. 1689.

[5] *Beitr. z. Klin. der Tuber.*, 1912, 23, p. 201; *München. med. Wchnschr.*, 1912, 59. p. 2560.

[6] *Ibid.*, p. 215.

[7] *Ibid.*, p. 223.

[8] *Ibid.*, p. 261.

TABLE I.

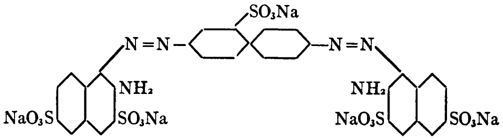
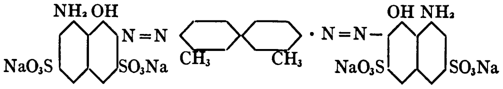
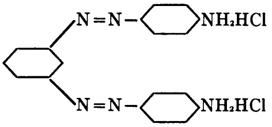
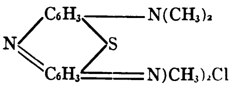
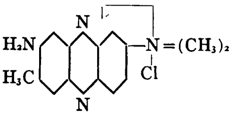
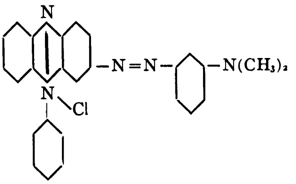
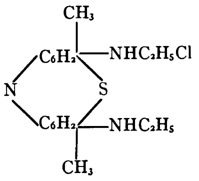
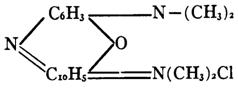
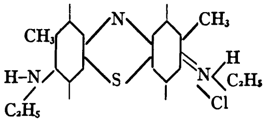
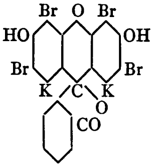
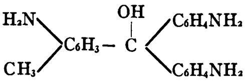
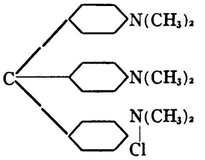
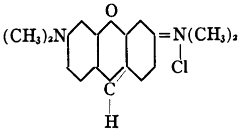
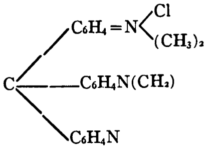
Name of Dye	Chemical Group	Chemical Structure
Trypan red............	An azo dye	 SO₃Na N=N N=N NH₂ NaO₃S SO₃Na NH₂ NaO₃S SO₃Na
Trypan blue...........	An azo dye	 NH₂ OH N=N NaO₃S SO₃Na CH₃ CH₃ OH NH₂ · N=N– NaO₃S SO₃Na
Bismarck brown.......	An azo dye	
Methylene blue........	Quinone imide Thiazin	
Neutral red...........	Quinone imide Eurhodine	
Janus green...........	Quinone imide Saffranin	
Brilliant cresyl blue.....	Quinone imide An oxazin dye	Structure not found
New methylene blue N..	Quinone imide A thiazin	
New methylene blue GG	Quinone imide An oxazin	
New methylene blue NX	Mixture of methylene blue 2B and methylene violet	Definite structure not found

TABLE 1—*Continued.*

Name of Dye	Chemical Group	Chemical Structure
New methylene blue R..	Chemical formula not found	
New methylene blue 3R	Chemical formula not found	
New methylene blue GB	Chemical formula not found	
New methylene blue NSS*	....	
Eosin.................	Phenyl methane	
Basic fuchsin..........	Phenyl methane	
Crystal violet..........	Phenyl methane	
Pyronin...............	Phenyl methane	
Pyrrhol blue...........	Phenyl methane	
Isamine blue...........	Phenyl methane Formula not found	Reactions very similar to pyrrhol blue

---

* A personal communication from the Casella Color Company informs me that the structure of new methylene blue NSS is that given for new methylene blue N, while the latter is a zinc double salt of the same formula.

TABLE 2.

Name of Dye	Water	Absolute Alcohol	Ether	Chloroform	Olive Oil	10 per cent HCl	10 per cent NaOH	10 per cent NH_3OH
Trypan blue	Freely soluble	Nearly insoluble	Insoluble	Insoluble	Insoluble	Soluble. No color change	Soluble. Reddish purple	Soluble. No color change
Trypan red	Freely soluble	Nearly insoluble	Insoluble	Insoluble	Insoluble	Soluble. No color change	Soluble. No color change	Soluble. Color deeper red
Bismarck brown	Freely soluble	Freely soluble	Slightly soluble	Insoluble	Slightly soluble	Soluble. No color change	Slightly soluble. Color yellow	Slightly soluble. Color yellow
Eosin A	Freely soluble	Freely soluble	Insoluble	Insoluble	Insoluble	Insoluble	Soluble. Color bluish	Soluble. Color yellowish red
Eosin blue	Freely soluble	Freely soluble	Insoluble	Insoluble	Insoluble	Insoluble	Soluble. No color change	Soluble. Color deep red
Erythrosin	Freely soluble	Freely soluble	Slightly soluble	Slightly soluble	Insoluble	Nearly insoluble	Soluble. Color purplish	Soluble. Color deep red
Pyrrhol blue	Freely soluble	Freely soluble	Slightly soluble	Slightly soluble	Slightly soluble	Slightly soluble. No color change	Insoluble	Soluble. No color change
Isamine blue	Freely soluble	Freely soluble	Slightly soluble	Insoluble	Insoluble	Slightly soluble. Color purplish	Slightly soluble. Color reddish	Soluble. No color change
Basic fuchsin	Freely soluble	Freely soluble	Insoluble	Slightly soluble	Very slightly soluble	Slightly soluble. Color yellowish	Insoluble	Slightly soluble
Crystal violet	Freely soluble	Freely soluble	Insoluble	Soluble	Soluble	Soluble. Color yellowish green	Insoluble	Slightly soluble. No color change
Ehrlich's rectified methylene blue	Freely soluble	Freely soluble	Insoluble	Slightly soluble	Insoluble	Soluble. No color change	Very slightly soluble. Color reddish	Soluble. No color change
Medicinally pure methylene blue	Freely soluble	Freely soluble	Insoluble	Insoluble	Insoluble	Soluble. No color change	Very slightly soluble. Color reddish	Soluble. No color change
Neutral red	Freely soluble	Freely soluble	Insoluble	Soluble	Insoluble	Soluble. Color bluish	Slightly soluble. Color yellow	Nearly insoluble. Color yellow
Janus green	Freely soluble	Freely soluble	Slightly soluble	Slightly soluble	Slightly soluble. Color pink	Soluble. Color bluish	Slightly soluble. Color reddish brown	Soluble. Color green
Brilliant cresyl blue	Freely soluble	Freely soluble	Insoluble	Slightly soluble	Insoluble	Soluble. Color reddish	Nearly insoluble	Soluble. Color reddish
Diamine schwarz	Freely soluble	Freely soluble	Slightly soluble	Slightly soluble. Color reddish	Insoluble	Insoluble	Slightly soluble. Color reddish	Freely soluble. Color bluish black
New methylene blue N	Freely soluble	Freely soluble	Insoluble	Slightly soluble	Insoluble	Soluble. No color change	Insoluble	Freely soluble. Color deep blue
New methylene blue GG	Freely soluble	Freely soluble	Slightly soluble. Color pink	Soluble. Color reddish purple	Soluble. Color red	Soluble. Color blue	Soluble. Greenish blue changing to brown	Soluble. Color greenish blue
New methylene blue NX	Freely soluble	Freely soluble	Slightly soluble	Freely soluble	Soluble. Color reddish	Soluble Color green changing to blue	Slightly soluble. Color blue	Freely soluble. Color deep blue
New methylene blue R	Freely soluble	Freely soluble	Insoluble	Freely soluble	Soluble. Color bluish red	Soluble. Color green changing to blue	Insoluble	Slightly soluble. Color blue
New methylene blue 3R	Freely soluble	Freely soluble	Insoluble	Freely soluble	Soluble. Color bluish red	Soluble. Color green changing to blue	Insoluble	Slightly soluble. Color reddish
New methylene blue GB	Freely soluble	Freely soluble	Insoluble	Freely soluble	Soluble. Color bluish red	Soluble. Color green changing to blue	Slightly soluble. Color blue	Soluble. Color blue
New methylene blue NSS	Freely soluble	Freely soluble	Insoluble	Freely soluble	Insoluble	Soluble. Color deep blue	Insoluble	Soluble. Color blue
Pyronin	Freely soluble	Freely soluble	Insoluble	Slightly soluble	Insoluble	Soluble. No color change	Insoluble	Slightly soluble. Color pink

Having constantly a chemotherapeutic aim in mind, the following problems must be solved in the study of each dye:

1. Will the dye penetrate the tubercle? This being a non-vascular structure, the question of penetration itself presents no inconsiderable difficulties.

2. Is it toxic to the animal? In a slow, chronic process like tuberculosis, a remedial agent must be one which is well borne by the patient and can be administered at intervals for long periods, if necessary. We can scarcely hope, as may be possible in acute infections, like many of the trypanosomiases, to complete the sterilizing and curative process by a single treatment.

3. Will the dye penetrate the tubercle bacillus? We can be sure of this only if the bacilli stain readily, clearly, and permanently.

4. If the dye penetrates the tubercle bacillus, does it kill it or injure its vitality or inhibit its growth?

5. If the dye does not itself kill the bacilli, can its structure be modified, can new side chains be introduced or can metals or other elements be substituted in its molecule to make it more bactericidal without at the same time interfering with its penetrating power and its innocuousness?

Table 1 gives a list of the dyes which have been used so far in the work with their chemical formulae as far as I have been able to find them.

The preparations of these dyes used by myself have been tested as to their solubilities in the ordinary reagents and as to their reactions to acids and alkalies. The results are given in Table 2. This is important since different preparations often differ in their solubilities and reactions.

I have tested the staining power of most of these dyes on fixed and unfixed tissues. Tissues were fixed in a chrome-formalin solution, imbedded in paraffin, and sectioned. The sections, after removal of paraffin, were stained with one per cent water solutions of the dyes, with the following results:

1. Trypan blue: diffuse light protoplasmic stain with much darker nuclei. No coarse blue granules.

2. Trypan red: diffuse protoplasmic stain, nuclei sometimes slightly darker. No granular stain.

3. Bismarck brown: sharp nuclear stain. Protoplasm light yellow.

4. Eosin A } diffuse protoplasmic stain.
5. Eosin B }
6. Erythrosin: diffuse protoplasmic stain. In some areas nuclei slightly darker.
7. Pyrrhol blue: nuclei darker than protoplasm.
8. Isamine blue: protoplasm diffusely stained. No differentiation of nuclei.
9. Basic fuchsin: nuclei differentiated.
10. Crystal violet: nuclei somewhat darker than protoplasm. Differentiation not very sharp.
11. Pyronin: slight nuclear stain.
12. Methylene blue (both rectified and medicinally pure): sharp nuclear differentiation.
13. Neutral red: sharp nuclear differentiation.
14. Janus green: sharp nuclear differentiation.
15. Brilliant cresyl blue: sharp nuclear stain.
16. New methylene blue N: very good, sharp nuclear stain in short time.
17. New methylene blue GG: good, sharp nuclear stain.

Fresh tissues from a guinea-pig were dropped while still warm in one per cent water solutions of the dyes, kept at room temperature, and removed at the end of 24 hours. Some portions were examined at once, while others were fixed, dehydrated, imbedded in paraffin, and sectioned. The latter method gave good results with a few of the dyes, but most of them were soluble in alcohol, so that the dye diffused through the tissue and no satisfactory results could be gained. Most of the results reported are, therefore, from the tissue immediately after removal from the stain. The results are as follows:

1. Trypan blue: no change in dye. Tissue deep blue. Sharp nuclear stain; no granular stain. Paraffin sections gave the same appearance.
2. Trypan red: no change in dye. Tissue deep red. Diffuse stain of protoplasm. No granular stain. In some areas nuclei were darker than protoplasm. Same appearance in the paraffin sections.
3. Erythrosin: diffuse protoplasmic stain. No nuclear differentiation. Much diffusion of stain in imbedding, but appearance in the main was the same in the sections.
4. Isamine blue: not stained at all.
5. Pyronin: protoplasm faintly stained; nuclei somewhat more deeply. Much diffusion of stain in imbedding.
6. Methylene blue: penetration not very good. Periphery well stained. Good nuclear differentiation; some diffusion of stain in imbedding, but nuclear stain was still quite sharp in sections.
7. Neutral red: penetration not very good. Sharp nuclear stain at periphery. In sections, stain more diffuse and differentiation of nuclei not so good.
8. Brilliant cresyl blue: penetration very poor. On surface, a good nuclear stain, still retained in the paraffin sections.

9. New methylene blue N: sharp nuclear stain at surface, but penetration was poor. Nuclear stain well preserved in sections.

10. New methylene blue GG: very little penetration. Nuclear stain at surface. Well preserved in sections.

Most of these dyes, as may be seen from these results, react to the nuclei as bases, either strongly or weakly, even though they have an acid chemical structure. A number of the dyes which have more recently been added to my list have not yet been subjected to the above tissue test.

In the animal experiments, one per cent water solutions of the dyes have been used in most of the experiments, although with the dyes which have been shown to be irritating in that concentration one-fourth of one per cent and even one-tenth of one per cent solutions have been employed. Most of the injections have been subcutaneous, but in some cases the dyes have been injected intraperitoneally. In all cases guinea-pigs which had been inoculated subcutaneously with human tuberculosis were used. The results of the animal experiments both as to the penetration of the tubercle and as to injury to the animal are briefly given in Tables 3 and 4. Since trypan blue has been more thoroughly tested than any of the other dyes, the work with this dye has been summarized by itself in Table 3, while Table 4 states briefly the work so far finished on the other dyes used.

Sixteen tuberculous guinea-pigs were injected for longer or shorter periods with one per cent aqueous solution of trypan blue, from 6 to 10 c.c. being given at each dose. This was always well borne and no local or general toxic or irritative effects were noticed in any of the animals, except that the pregnant pigs always aborted, and most of them died shortly after the abortion. This may be due to the concentration of the dye in the placenta, which was noted by Goldmann and which I also have observed, but it is not in agreement with his observation on mice, as he says that these pass normally through several pregnancies, with no ill effects from the dye. In nearly every case, the smaller tubercles of lungs, liver, and spleen have taken the stain and are usually sharply contrasted with the surrounding tissue, which is either unstained or less deeply stained than the tubercles. The larger necrotic areas

TABLE 3.

ANIMAL EXPERIMENTS WITH TRYPAN BLUE.

Number	Method of Injection	Number of Injections	Concentration of Dye	Age of Infection	Autopsy Findings	Result of Dye Injections on Tubercle
1.....	Subcutaneous	3	1 per cent	59 days	Severe local and general tuberculosis	Tubercles all blue. Central mass unstained
2.....	Subcutaneous	5	1 per cent	90 days	Severe local and general tuberculosis	Tubercles all blue. Pus blue. No stained bacilli. No growth on agar tubes
3.....	Subcutaneous	3	1 per cent	107 days	Severe local, no general tuberculosis	Tuberculous gland blue. Caseous center blue. No stained bacilli
4.....	Subcutaneous	4	1 per cent	15 days	General miliary tuberculosis with peritonitis	Tubercles blue, but not deeper than surrounding tissue
5.....	Subcutaneous	8	1 per cent	126 days	Severe local and general tuberculosis	All tubercles bright blue. Caseous and softened centers bright blue
6.....	Subcutaneous	1	1 per cent	92 days	Severe local and general tuberculosis. Large necrotic spleen	All tubercles blue. Necrotic areas especially blue. Normal spleen unstained
7.....	Subcutaneous	4	1 per cent	126 days	Severe local and general tuberculosis	All tubercles blue. Liver and spleen tubercles especially blue
8.....	Subcutaneous	6	1 per cent	72 days	Local glandular and spleen tuberculosis	All tubercles blue. Caseous centers deep blue. No distinctly stained bacilli, but stained granules
9.....	Subcutaneous	1	1 per cent	69 days	Severe local, no general tuberculosis	Periphery of tuberculous gland blue. Center not stained
10.....	Subcutaneous	3	1 per cent	81 days	Severe local, no general tuberculosis	Tuberculous glands blue. Caseous centers blue. Bacilli not stained
11.....	Subcutaneous	4	1 per cent	100 days	Severe local, slight general tuberculosis	All tubercles blue. Caseous centers blue. No stained bacilli found
12.....	Subcutaneous	4	1 per cent	100 days	No local, no general tuberculosis	Distribution of dye as in normal pigs
13.....	Subcutaneous	2	1 per cent	119 days	Severe local and general tuberculosis	All tubercles blue. Caseous centers blue. Necrotic areas very blue
14.....	Intraperitoneal	1	1 per cent	90 days	Severe local and general tuberculosis	All tubercles blue
15.....	Intraperitoneal	1	1 per cent	93 days	Severe local and general tuberculosis	All tubercles blue
16.....	Intraperitoneal	1	1 per cent	93 days	Severe local and general tuberculosis	All tubercles very blue. Very little stain in normal tissue

so frequently present in the liver and spleen of the tuberculous guinea-pig take the stain with especial avidity. The large caseous and softened lymph glands always show a blue periphery, and if the injections have been repeated a number of times (*hochgetrieben*, as Goldmann calls it) the caseous and softened centers are also deep blue, and often deep blue pus exudes from the tubercle for some weeks before death. Distinctly blue stained tubercle bacilli have never been noted, either in this blue pus or in the smaller tubercles, although at times deeply blue stained granules are seen which appear to be in bacilli, and these may retain a blue stain to some extent even after the bacilli have been stained with carbol fuchsin. The staining of tubercle bacilli in the body with trypan blue has not, however, been satisfactorily proven. Cultures from the blue stained pus on glycerin agar have been made several times, but no growth has developed, and animal inoculations from this pus have not been made.

On microscopic examination, the smaller tubercles in the trypan blue sections show a peripheral zone of cells filled with coarse deep blue granules, cells like those found everywhere in the interstitial tissue and which are probably the cells described by Goldmann as *Pyrrholzellen*, although the granules are somewhat larger and more irregular in size than in the normal cells.

Central to this is a zone which shows very little blue stain, but which, in sections stained with sudan III, exhibits many red-stained droplets and granules (fat). The center of the tubercle is blue, more or less diffuse or filled with fine granules with a few coarser ones intermingled. This appearance is so different from any seen in the normal tissues stained with trypan blue that such an area can readily be picked out under a very low magnification. The color is bright blue as contrasted with the dark blue or blue black of the intracellular granules normally dyed by this stain when injected into the living animal. Tuberculous giant cells, when present, show a diffuse pale blue center with somewhat darker blue nuclei around the periphery. Goldmann describes similar findings in his mice inoculated with avian and also with bovine tuberculosis. He states that through the bacterial invasion and through the influence of the bacilli, the cells suffer an injury which expresses itself in the gradual loss of granular structure. The vital dye, anchored by the granules, after solution of the granules, first appears in coarser granules and later diffuse in the cell protoplasm and then finally disappears. The cell injury, he states, is only partial, since the nuclei remain unchanged and stainable and showing no degenerative phenomena even after perfect disappearance of the granules. He thinks that with the loss of granular structure, the cells lose their ameboid motility, and other cells collect around them forming larger and smaller cell conglomerates in which all transition stages

TABLE 4.

ANIMAL EXPERIMENTS WITH DYES.

Number	Dye Used	Concentration	Method of Injection	Number of Injections	Time of Action of Dye	Age of Infection	Effect of Dye during Life	Autopsy Findings	Postmortem Dye Findings
1.......	Janus green....	1 in 1,500	Into aorta after bleeding	1	None	54 days	None	Local and general tuberculosis	Tubercles unstained. Normal tissues well stained
2.......	Janus green	¼ per cent	Subcutaneous	4	8 days	3 months	Infiltration and sloughing	Local, no general tuberculosis	Tubercle unstained. Other tissues little stained
3.......	Pyronin.......	¼ per cent	Subcutaneous	3	6 days	?	No ill effects	Severe local and general tuberculosis	Tubercles unstained. Other tissues unstained
4.......	Neutral red	¼ per cent and 1 per cent	Subcutaneous	6	16 days	?	No ill effects	Local, no general tuberculosis	Tubercles very slightly stained
5.......	Neutral red	1 per cent	Subcutaneous	4	56 days	65 days	No ill effects	Local and general tuberculosis	Tubercles in lung very faintly stained
6.......	Neutral red	1 per cent	Subcutaneous	7	73 days	107 days	No ill effects	Local, no general tuberculosis	Tubercles unstained
7.......	Neutral red	1 per cent	Subcutaneous	10	25 days	?	No ill effects	Local, no general tuberculosis	Periphery of caseous glands stained. Center unstained
8.......	Erythrosin	1 per cent	Intra-aortic	1	None	71 days	None	Severe local and general tuberculosis	Tubercles and necrotic areas unstained. Other tissues bright red
9.......	Rectified methylene blue	1 in 2,000	Intra-aortic	1	None	60 days	None	Local and general tuberculosis	Tubercles unstained. Other tissues well stained
10.......	Rectified methylene blue	⅓ per cent	Intra-aortic	1	None	46 days	None	Local and general tuberculosis	Tubercles unstained. Other tissues stained
11.......	Rectified methylene blue	¼ per cent	Subcutaneous	4	7 days	?	Infiltration and sloughing	Slight local, severe general tuberculosis	Tubercles unstained. Other issues unstained
12.......	Rectified methylene blue	¼ per cent and 1 per cent	Subcutaneous	6	14 days	?	Infiltration and sloughing	Local and general tuberculosis	Tubercles well stained after oxidation of dye
13, 14, 15, 16.....	Medicinally pure methylene blue	1 per cent	Subcutaneous	2	5 to 8 days	?	Much infiltration	No tubercles found	Tissues blue
17.......	Medicinally pure methylene blue	¼ per cent	Subcutaneous	4	7 days	?	Infiltration and sloughing	Local and general tuberculosis	Tubercles very little stained

18.......	Medicinally pure methylene blue	½ per cent	Subcutaneous	1	2 days	?	Some infiltration	Local, no general tuberculosis	No stain anywhere
19.......	New methylene blue N	½ per cent and 1 per cent	Subcutaneous	9	28 days	?	Slight infiltration. Well borne	Local and general tuberculosis	Internal tubercles blue after oxidation. Bacilli not stained
20.......	New methylene blue GG	½ per cent and 1 per cent	Subcutaneous	7	23 days	?	Slight infiltration. In main well borne	Local and general tuberculosis	All tubercles blue after oxidation. No stained bacilli
21.......	Methylene blue of U.S. Pharmacopeia	1 per cent	Subcutaneous	4	8 days	?	Marked infiltration and sloughing	Local and general tuberculosis	Internal tubercles stained. No stained bacilli
22.......	Same as 21	1 per cent	Subcutaneous	1	1 day	?	No ill effects	Local and general tuberculosis	Internal tubercles deep blue after oxidation. No stained bacilli
23.......	Trypan red	1 per cent	Subcutaneous	7	7 days	?	No ill effects	Local and general tuberculosis	All tubercles stained deep red; also pus of caseous glands
24.......	Trypan red	1 per cent	Intraperitoneal	1	6 days	82 days	No ill effects	Local and general tuberculosis	All tubercles stained. No stained bacilli
25.......	Trypan red	1 per cent	Intraperitoneal	1	10 days	92 days	No ill effects	Local and general tuberculosis	All tubercles stained. No stained bacilli
26.......	Trypan red	1 per cent	Intraperitoneal	1	7 days	87 days	No ill effects	General glandular tuberculosis. Liver, spleen, and lungs not involved	Periphery of all tuberculous glands red. Centers unstained
27.......	Bismarck brown	1 per cent	Subcutaneous	3	6 days	?	None	No tuberculosis	No stain anywhere
28.......	Bismarck brown	1 per cent	Subcutaneous	3	7 days	?	No ill effects	No tuberculosis	No dye anywhere

may be seen. He also notes that with the change in the granular structure of the cell, and especially after the granules have entirely disappeared, a fat reaction is given by appropriate dyes.

The large necrotic areas of liver and spleen also have the bright blue diffuse appearance seen in the true tubercle. These areas take up the stain more quickly and readily even than the true tubercles.

The manner in which the normal tissues react to trypan blue when introduced during life has been so well described in the various communications by Goldmann and others that no detailed description is needed here. It is sufficient to say that in nearly all organs and in the skin, he found certain cells of the interstitial tissue which take up the stain in granular form, these granules being rendered visible by the dye, but being invisible when the dye has not been used. He calls these cells *Pyrrholzellen*. It is impossible to avoid the thought that this stain in normal tissues may be due to a phagocytic action, especially as the cells most active in taking up the dye are active phagocytes toward other foreign substances. Goldmann, however, is very sure, from his observations, that the action is not phagocytosis, and Gross asserts that it is due to a special activity of the cells, while Schulemann thinks that the granules are *Reactionskörper* formed by the entrance of the dye into the cells. Without discussing this question further at this time, it may be said that the staining of the tubercle and of the necrotic tissue is quite different from that of the normal tissue, responding to quite a different law. While the nuclei of normal living cells probably never stain with trypan blue, the nuclei of the tuberculous giant cells are especially stained; also the diffuse, hyaline appearing stain of the protoplasm is probably rarely, if ever, found in the normal tissue vitally stained by trypan blue. It may be said, however, that this stain is not specific for the tubercle. Numerous investigators have demonstrated that injured kidney cells show nuclear stain and diffuse protoplasmic stain with trypan blue. Goldmann states that in experimentally produced liver necrosis a similar change in the normal reaction to the dye occurs. Brown and Evans observed a similar stain of cells of the central nervous system in experimental anterior poliomyelitis, and in several guinea-pigs I caused necrosis of muscle by injection of formalin and found the muscle, which normally takes up no or very little trypan blue, staining deeply and diffusely blue. The same phenomenon was noted by Ribbert[1] as to the action of carmine granules introduced during life; the usual granular appearance being lost and the injured cells showing diffusely stained protoplasm with darker nuclei.

Even though not specific for the tubercle, however, an important point is gained in that we have in trypan blue a dye which penetrates the tubercle quite easily, and remains in it for a considerable period. I have found tubercles well stained in animals which were killed as late as nine days after the last injection, while I have also found them well stained in animals killed or dying as early as 12 hours after a single injection. We have, then, in trypan blue a dye which penetrates the tubercle easily and quickly and remains for some time fixed in the tubercle. It is also practically innocuous for the animal, as I have in many cases given 8 to 10 c.c. of one per cent solution several times a week for weeks with no noticeable ill effect, either to the general condition or locally at the point of injection. It is one of the dyes, which, like trypan red, was found by Ehrlich and his workers to have a marked trypanocidal power over some kinds of trypanosomes. It is therefore worthy of careful study with relation to its bactericidal action in tuberculosis. The results of my work in this direction will be given more in detail a little later in this report. It may be said that the animals listed in Table 3 received no injections of the dye until very late in the disease, and very little attention was at this time paid to curative properties, most of the animals being killed, without waiting to see how long they might have lived under the treatment. My whole attention at this stage of the work was given to the penetrating and staining power of the dye.

Table 4 gives a series of experiments with various dyes as to their penetrating power and their toxicity for the animal. From this table, although in general the number of experiments with each dye is small, the following facts are noted:

1. Janus green is unpromising for treatment because it causes deep sloughing if used subcutaneously for any time and does not penetrate the tubercle.

---

[1] *Ztschr. f. allg. Physiol.*, 1904, 4, p. 201.

2. Pyronin: the dye is well borne but rapidly excreted and seems not to penetrate the tubercle. Or, if it does, it passes out of it quickly, since the pig was killed within 12 hours after an injection and no stain, or very little, was found in the tubercle.

3. Neutral red: this dye is well borne by the animal but shows very little, if any, power to penetrate the tubercle. Further experiments will be made with this dye.

4. Erythrosin: only one experiment and that a post-vital, intracardiac injection. In this the stain did not penetrate the tubercles. As the dye seems to be quite well borne and is in other respects favorable, further experiments and a later report will be made on this dye.

5. Methylene blue: several preparations have been used:

a) Ehrlich's rectified methylene blue, which in post-vital, intracardiac injection did not penetrate the tubercle. In subcutaneous, repeated injections, it penetrated the smaller tubercles of lungs, liver, and spleen, but not to the softened centers of caseous glands. In the small tubercles, the dye was reduced to the leukobase, so that no color appeared until after exposure to air and sometimes not until after the tissue was placed in ammonium molybdate solution. Then the tubercles became intensely blue and remained so during the process of dehydrating and imbedding in paraffin.

In a one-fourth of one per cent solution, injected subcutaneously, it caused much induration and some sloughing, but in weaker solutions and in small amounts it is well borne.

b) Methylene blue—medicinally pure: the results of the experiments finished have been about the same as with Ehrlich's rectified methylene blue.

c) Methylene blue of the U.S. Pharmacopeia: results were the same as with other methylene blues, but local reaction was not so severe.

A larger number of tuberculous guinea-pigs are now being systematically treated with this dye, a report of which will be published later.

d) New methylene blue N.

e) New methylene blue GG.

Both (*d*) and (*e*) give the same general results as the ordinary methylene blues and are about as well borne by the animals.

Further experiments with these dyes are in progress as well as with other new methylene blues.

6. Trypan red: this is the dye first shown by Ehrlich and Shiga to have the power to destroy trypanosomes in the animal body. My experiments, while not so numerous as those with trypan blue, show that the dye readily penetrates the tubercle, although not quite so readily as does trypan blue. No ill effects have been observed in guinea-pigs from the use of this dye. My results with this dye have practically agreed with the experiment reported by Lewis on the rabbit.

7. Bismarck brown, while apparently innocuous to the animal, is rapidly excreted, and I could find no trace of the dye in any of the organs twelve hours after the last injection. The guinea-pigs used proved non-tuberculous, so that the penetration of the tubercle was not tested.

Other dyes are now being tested, and report of their influence will be published later. Other dyes and dye modifications are being worked out. So far, then, we find that trypan blue, trypan red, to a slight extent neutral red and pyronin, isamine blue, as tested by Lewis, and pyrrhol blue, which acts in all respects like isamine blue, Ehrlich's rectified methylene blue, Pharmacopeial methylene blue, medicinally pure methylene blue, new methylene blue N, and new methylene blue GG may penetrate the tubercles with less or greater ease and are borne for long periods in larger or smaller doses with no great inconvenience.

The next question of importance then is: Do these dyes penetrate the tubercle bacillus? Do they stain the dead, fixed bacillus? Do they stain the living bacillus *in vitro?* Do they stain the bacillus in the tubercle? Numerous tests have been made with the various dyes to determine their power to stain the tubercle bacilli. It may be stated that human tubercle bacilli grown on glycerin agar have been used almost exclusively. The following methods have been tested for staining: (1) inoculating with tubercle culture tubes of glycerin agar to which had been added varying quantities of the dyes; (2) inoculating glycerin agar tubes with tubercle bacillus cultures, and, after these were somewhat grown, filling up the tube with one per cent water solutions of the dyes; (3) adding a small amount of suspension of tubercle bacilli to the dye, and, after certain intervals of time, washing, centrifuging, and making smears; (4) ordinary smears on slides, fixed by heat, were placed in one per cent water solutions of the dyes, sometimes hot and sometimes cold, and examined at the end of different periods of time. The final results in most cases were obtained at the end of 20 to 24 hours at 39° C. temperature. This of course does not apply to the growth experiments on stained glycerin agar, as in these the macroscopic staining was noted at the end of 24 hours, but the microscopic appearance only at the end of a growth period of four to six weeks. As the results of all these tests corresponded in a general way, it will be sufficient to state the general results without reporting the various experiments in detail.

1. Trypan blue gives a good mass stain, but individual bacilli are but faintly stained, and most of the individuals not at all. As this dye had proved so promising for use in animal experiments, very special efforts were made to strengthen its staining power and to obtain a good individual stain of the bacilli. The following methods were used:

a) Heat-fixed smears of tubercle bacilli were stained 24 hours in cold one per cent water solution of trypan blue. Groups of bacilli and some few individuals were faintly stained.

b) Heat-fixed smears of tubercle bacilli remained 24–48 hours in one per cent water solution of trypan blue at 39° C. Stain was slightly deeper both in groups and in individuals, and granules were stained dark in some bacilli. Most of the individuals, however, remained unstained.

c) Smears were stained as in (*b*) and then washed in 20 per cent formalin. A heavy precipitate formed, but the stain was not improved.

d) Smears were fixed in absolute methyl alcohol instead of by heat and staining attempted by various methods, but results were no better than in heat-fixed smears.

e) Potassium hydroxid was applied to the smear before staining, but the results were no better.

f) An agar slant culture of human tubercle bacilli was covered with one per cent water solution of trypan blue and left in the incubator for 24 hours. The dye was then poured off, the slant washed, and a portion of the culture spread on slides. The stain seemed somewhat clearer and darker, but still not very clear. Not all bacilli were stained and even the best stained still appeared somewhat shadowy.

g) Five per cent of phenol was added to the dye and this was used on the agar slant as in (*f*) and the tubes were left in the incubator 24–48 hours. The stain appeared darker and more granules were seen.

h) Formalin was used in the same way as phenol under (*g*) and with about the same results.

i) Potassium hydroxid was used in same way as phenol in (*f*) but the stain was not at all improved.

j) All the above methods were used on strong thick emulsions of tubercle bacilli, afterward washing and centrifuging. The results were not materially different from those obtained from agar slants. From all these tests, it may be stated that trypan blue readily stains a mass of bacilli and perhaps also the outer covering of some individuals, but does not readily or satisfactorily penetrate individual bacilli.

2. Trypan red also gives a fairly good mass stain, but the individual stain is even fainter and more unsatisfactory than that of trypan blue.

3. Methylene blue: five modifications of this dye have been tested as to their power to stain tubercle bacilli—Ehrlich's rectified methylene blue, medicinally pure methylene blue, the methylene blue of the U.S. Pharmacopeia, and the new methylene blue N and new methylene blue GG. All these preparations stain masses of tubercle bacilli quickly and intensely—all readily stain individual bacilli clearly, deeply, and permanently. This latter action is more intense on the unfixed bacilli of the emulsion or agar slant than on the smear fixed by heat. But even in the latter there is no question that the individual bacilli are well and permanently stained. Granules within the bacilli are often more intensely stained than the rest of the organism.

4. Eosin A, eosin blue, and erythrosin all stain both masses of bacilli and single bacilli clearly and permanently. These three dyes were used always in one per cent water solution, and, while they stained the smears fixed by heat very well, they all acted most intensely on the fresh, living, or unfixed organism. Erythrosin, of the three preparations, stained the bacilli most intensely, and eosin A least.

5. Neutral red gave a good mass stain, but a very faint individual stain.

6. Pyronin also gave good mass stain but very faint individual stain, or none at all.

7. Janus green also gave a fair mass stain but practically no stain of individual tubercle bacilli.

8. Gentian violet stains quickly and intensely both masses of bacilli and single organisms.

9. Basic fuchsin also stains very deeply both masses of tubercle bacilli and individuals.

10. Brilliant cresyl blue has very little power to stain either masses of tubercle bacilli or single organisms.

11. Bismarck brown stains masses of bacilli very well, but usually dyes individual organisms but faintly. Occasionally I have had preparations in which the stain was clearer and deeper than usual.

12. Isamine blue and pyrrhol blue are much like trypan blue in their good mass staining with faint, weak individual staining.

So it may be seen that practically all the dyes studied stain masses of tubercle bacilli well in a very few minutes, but it is much more difficult to penetrate the individual organism and color its interior. Most of the dyes seem simply to form some kind of combination with the bacillary membrane and not to be able to pass through it. Only gentian violet, basic fuchsin, erythrosin, and the eosins, the different methylene blue preparations, and the new methylene blues may be said to stain uniformly and satisfactorily the interior of the tubercle bacilli, and this, in the question of the therapeutic value of a substance, is of prime importance.

In connection with the penetration and staining of tubercle bacilli by these various dyes, the question of their bactericidal power is of very great importance. The dyes used throughout these experiments were first tested for their bactericidal or their inhibiting action *in vitro* on a number of ordinary organisms. Several methods were used to test the bactericidal or inhibiting action of the dyes on the various organisms. The bacteria used have been *B. coli*, *B. typhosus*, *B. dysenteriae* (Shiga), *B. prodigiosus*, *B. pyocyaneus*, *B. subtilis*, *Staphylococcus aureus*, and an acid-fast organism supposed to be an old attentuated human tubercle culture. Often the growth was reduced in amount and the colonies in number, even though growth took place. The methods used have been: (1) a somewhat modified Anderson-McClintock method, in which cultures were made both in broth and on agar slants, the latter bringing out the quantitative relation much better than the broth; (2) adding definite amounts of dye to agar tubes and then inoculating and watching them; (3) broth cultures of the different organisms were grown at room temperature for 72 hours; then 10 drops of one per cent water solution of the dye were added to each tube containing 10 c.c. of a moderately thick bacterial emulsion; after certain definite times, a loopful from each tube was streaked on agar slants. Table 5 gives briefly the results of one of the experiments, and, as all the results agreed fairly well, this may stand as the report of this work.

TABLE 5.

GROWTH EXPERIMENTS OF ORDINARY BACTERIA ON STAINED AGAR. TWO DROPS OF ONE PER CENT SOLUTION OF DYE TO EACH TUBE OF AGAR

Dyes Used	*B. tuberculosis**	*Staphylococcus aureus*	*B. subtilis*	*B. typhosus*	*B. pyocyaneus*	*B. dysenteriae* (Shiga)	*B. prodigiosus*	*B. coli*
Basic fuchsin....	–	+	–	+++	+++	+	+++	+++
Bismarck brown.	–	++	+++	++	++	+	+++	+++
Trypan red.....	+++	+++	+++	+++	+++	+	+++	+++
Trypan blue.....	+++	+++	+++	+++	+++	+	+++	+++
Isamine blue....	+++	+++	+++	+++	+++	+	+++	+
Ehrlich rectified methylene blue	–	–	–	+	+++	+	+	+
Medicinally pure methylene blue	–	–	–	+	+++	+	+	+
New methylene blue N.......	–	–	–	+++	+++	+	+	+++
New methylene blue GG......	–	–	–	+++	+++	+	+	+
Eosin A........	+	+++	+++	+++	+++	+	+++	+
Eosin blue......	+++	+++	–	+++	+++	+	+++	++
Erythrosin......	+	+++	+++	+	+++	+	+++	+++
Pyronin........	+	–	–	+++	+++	+	+++	+++
Janus green.....	–	–	–	+++	+++	+	+++	+
Neutral red.....	+	–	+++	+++	+++	+	+	+++
Gentian violet...	+	–	–	+++	+++	+	+++	+++
Pyrrhol blue.....	+++	+++	+++	+++	+++	+	+++	+++
Brilliant cresyl blue.........	+	–	–	+++	+++	+	+	+
Control........	+++	+++	+++	+++	+++	++	+++	+++

* The organism in column one was an acid-fast organism, supposed to be an old, attenuated tubercle bacillus culture, but its history was not exactly known. It grows rapidly and luxuriantly, and produces abscesses in guinea-pigs. These abscesses are acute or at least develop much more rapidly than tuberculous glands, and other pigs in the same cage are infected.

– Signifies no growth.

+ Signifies little growth.

++ Signifies moderate growth.

+++ Signifies luxuriant growth.

From this table it may be seen that the questionable tubercle bacillus was killed or inhibited by basic fuchsin, bismarck brown, Ehrlich's rectified methylene blue, medicinally pure methylene blue, new methylene blue N, new methylene blue GG, and Janus green, and its growth was diminished by erythrosin, eosin, pyronin, gentian violet, brilliant cresyl blue, and neutral red. *Staphylococcus aureus* showed no growth in the tubes containing any of the methylene blues, pyronin, Janus green, gentian violet, brilliant cresyl blue, and neutral red. *B. subtilis* failed to develop in tubes containing the five methylene blues, pyronin, Janus green, gentian violet, and eosin blue. While other organisms were much diminished in their growth by certain dyes, none of them showed an entire absence of growth. While these results do not agree in all details with those obtained by Churchman in his work on the bactericidal action of gentian violet, they verify his statement that gentian violet and also other dyes not mentioned by him have a certain selective bactericidal action. It should be noted here that this action noted in Table 5 is partly inhibitory, especially so far as the methylene blues are concerned. In the experiment in which 10 drops of the dyes were added to thick broth cultures, none of the cultures were killed by the methylene blue even after 24 hours' exposure, although the growth was diminished in some cases. In this experiment, no growth of the acid-fast organism developed after exposure 24 hours to bismarck brown, erythrosin, pyronin, Janus green, eosin, while the basic fuchsin, trypan blue, and gentian violet tubes gave from one to three colonies. *Staphylococcus aureus* failed to develop after 24 hours' exposure to basic fuchsin, pyronin, Janus green, and eosin. *B. subtilis* showed some growth on all the tubes, but only three to five colonies in the tubes after exposure 24 hours to erythrosin, pyronin, Janus green, eosin, and gentian violet. The slight differences in these results are perhaps due to the fact that in the broth culture after three days' growth, many larger clumps had formed which were not easily penetrated by the methylene blues and some of the other dyes.

An interesting fact was noted in these experiments—that all the methylene blues and brilliant cresyl blue are reduced by the growth of certain organisms, especially in this experiment, by *B. typhosus*, *B. coli*, *B. prodigiosus*, and *B. pyocyaneus*. In all these tubes the color was quickly restored by the addition of hydrogen peroxid.

The bactericidal action of these dyes on the tubercle bacillus outside the animal body has been tested several times on stained agar tubes to determine whether tubercle cultures would develop

TABLE 6.

BACTERICIDAL ACTION OF TRYPAN BLUE ON TUBERCLE BACILLI, AS TESTED BY ANIMAL INOCULATION.

Dye Concentration	Quantity of Dye	Quantity of Bacillus Emulsion	Time of Exposure	Animal Used	Time after Injection before Tubercle Was Observed	Time after Injection before Death	Autopsy Findings
3 per cent.......	5 c.c.	0.1 c.c.	5 minutes	Guinea-pig	15 days	78 days	Local and general tuberculosis
2 per cent.......	5 c.c.	0.1 c.c.	5 minutes	Guinea-pig	15 days	85 days (then killed)	Local and general tuberculosis
1 per cent.......	5 c.c.	0.1 c.c.	5 minutes	Guinea-pig	15 days	84 days	Local and general tuberculosis
0.1 per cent.....	5 c.c.	0.1 c.c.	5 minutes	Guinea-pig	15 days	81 days	Severe local and general tuberculosis
Distilled water (control)......	5 c.c.	0.1 c.c.	5 minutes	Guinea-pig	15 days	46 days (killed)	Marked local and general tuberculosis
3 per cent.......	5 c.c.	0.1 c.c.	60 minutes	Guinea-pig	15 days	68 days	Severe local and general tuberculosis
2 per cent.......	5 c.c.	0.1 c.c.	60 minutes	Guinea-pig	15 days	77 days	No autopsy
1 per cent.......	5 c.c.	0.1 c.c.	60 minutes	Guinea-pig	15 days	83 days	Marked local and general tuberculosis
0.1 per cent.....	5 c.c.	0.1 c.c.	60 minutes	Guinea-pig	15 days	77 days	Severe local and general tuberculosis
Distilled water (control)......	5 c.c.	0.1 c.c.	60 minutes	Guinea-pig	38 days	53 days	Slight local, severe general tuberculosis
3 per cent.......	5 c.c.	0.1 c.c.	20 hours	Guinea-pig	15 days	47 days (killed)	Severe local and general tuberculosis
2 per cent.......	5 c.c.	0.1 c.c.	20 hours	Guinea-pig	15 days	74 days	Severe local and general tuberculosis
1 per cent.......	5 c.c.	0.1 c.c.	20 hours	Guinea-pig	15 days	95 days (killed)	Severe local and general tuberculosis
0.1 per cent.....	5 c.c.	0.1 c.c.	20 hours	Guinea-pig	15 days	78 days	Severe local and general tuberculosis
Distilled water (control)......	5 c.c.	0.1 c.c.	20 hours	Guinea-pig	15 days	77 days	No autopsy
3 per cent.......	5 c.c.	0.1 c.c.	44 hours	Guinea-pig	15 days	53 days	Severe local and general tuberculosis
2 per cent.......	5 c.c.	0.1 c.c.	44 hours	Guinea-pig	25 days	78 days	Severe local and general tuberculosis
1 per cent.......	5 c.c.	0.1 c.c.	44 hours	Guinea-pig	25 days	85 days (killed)	Severe local and general tuberculosis
0.1 per cent.....	5 c.c.	0.1 c.c.	44 hours	Guinea-pig	25 days	92 days (killed)	Severe local and general tuberculosis
Distilled water (control)......	5 c.c.	0.1 c.c.	44 hours	Guinea-pig	25 days	95 days (killed)	Severe tuberculous lymph adenitis. Liver and spleen not involved

in media colored with the dye in question. On account of the difficulty of securing uniform results by this method on so freakish an organism as the tubercle bacillus, inoculations have been made into guinea-pigs of material which had been subjected to the action of the dye for varying times. Trypan blue has been very thoroughly tested as to its bactericidal power over tubercle bacilli, and Table 6 gives in brief form the results of these tests. From this table, it may readily be seen that this dye has no appreciable power to kill these bacilli. In this experiment, as well as in those with the other dyes, the emulsion of tubercle bacilli was filtered and smears showed that there were no larger clumps, the bacilli being in the main single. This condition is of course most favorable for killing the organisms. One-tenth cubic centimeter of this filtered emulsion was added to five cubic centimeters of one per cent solution of

TABLE 7.

BACTERICIDAL OR INHIBITORY ACTION OF DYES ON TUBERCLE BACILLI.
A. *In vitro.*

Dyes Used	Amount of 1 Per Cent Dye Solution to 10 c.c. Agar Tube	Staining Result	Growth Result
Trypan blue	2 drops to agar tube 4 drops to agar tube	Good mass stain No individual stain	Good growth
Trypan red	2 drops to agar tube 4 drops to agar tube	Good mass stain No individual stain	Growth good
Rectified methylene blue	2 drops to agar tube 4 drops to agar tube	Good mass stain Good individual stain	No growth
Medicinally pure methylene blue	2 drops to agar tube 4 drops to agar tube	Good mass stain Good individual stain	No growth
Pharmacopeial methylene blue	2 drops to agar tube 4 drops to agar tube	Good mass stain Good individual stain	No growth
New methylene blue N	2 drops to agar tube 4 drops to agar tube	Good mass stain Good individual stain	No growth
New methylene blue GG	2 drops to agar tube 4 drops to agar tube	Good mass stain Good individual stain	No growth
Brilliant cresyl blue	2 drops to agar tube 4 drops to agar tube	Good mass stain Fair individual stain	No growth
Gentian violet	2 drops to agar tube 4 drops to agar tube	No stain (1) Good mass and fair individual stain (2)	Good growth (1) No growth (2)
Pyronin	2 drops to agar tube 4 drops to agar tube	Good mass stain (1) No individual stain (2)	Good growth (1) Slight growth (2)
Neutral red	2 drops to agar tube 4 drops to agar tube	Only mass stain in both tubes	No growth in either tube
Janus green	2 drops to agar tube 4 drops to agar tube	Only mass stain in both tubes	No growth in either tube
Eosin A	2 drops to agar tube 4 drops to agar tube	Good mass stain Fair individual stain	No growth
Blue eosin	2 drops to agar tube 4 drops to agar tube	Good mass stain Fair individual stain	No growth
Erythrosin	2 drops to agar tube 4 drops to agar tube	Good mass stain Good individual stain	No growth
Bismarck brown	2 drops to agar tube 4 drops to agar tube	Only mass stain in both tubes	No growth
Pyrrhol blue Isamine blue	2 drops to agar tube 4 drops to agar tube	Only mass stain in all tubes	Good growth in all tubes

TABLE 7—*Continued.*

BACTERICIDAL OR INHIBITORY ACTION OF DYES ON TUBERCLE BACILLI.
B. *In vivo.*

Dyes Used	Time of Exposure	Staining Results	Results in Animal
Trypan blue 0.1 per cent to 3 per cent water solution	5 minutes to 44 hours	Good mass stain Faint individual stain	All animals developed tubercles in 15 to 25 days
Trypan red. 1 per cent water solution	24 hours	Good mass stain Faint individual stain	Developed tubercle in 19 days
Rectified methylene blue. 1 per cent water solution	24 hours	Both mass and individual stain good	No tubercle had developed after 55 days
Medicinally pure methylene blue. 1 per cent water solution	24 hours	Both mass and individual stain good	No tubercle had developed after 55 days
Pharmacopeial methylene blue 1 per cent water solution	24 hours	Both mass and individual stain good	Tubercle observed in 25 days
New methylene blue N. 1 per per cent water solution	24 hours	Mass and individual stain excellent	Tubercle observed in 25 days
New methylene blue GG. 1 per cent water solution	24 hours	Mass and individual stain good	Tubercle observed in 25 days
Brilliant cresyl blue. 1 per cent water solution	24 hours	Mass and individual stain fair	No tubercle after 55 days
Gentian violet. 1 per cent water solution	24 hours	Both mass and individual stain excellent	Tubercle observed in 35 days
Pyronin. 1 per cent water solution	24 hours	Only mass stain	Tubercle found in 19 days
Neutral red. 1 per cent water solution	24 hours	Only mass stain	Tubercle developed after 47 days
Janus green. 1 per cent water solution	24 hours	Only mass stain	Tubercle developed in 25 days
Eosin A	Not tested		
Eosin blue. 1 per cent water solution	24 hours	Good mass and individual stain	Tubercle developed in 25 days
Erythrosin. 1 per cent water solution	24 hours	Mass and individual stain excellent	Tubercle developed in 25 days
Bismarck brown. 1 per cent water solution	24 hours	Only mass stain	No tubercle after 55 days
Pyrrhol blue	Not tested		
Isamine blue			

dye, and at the end of the desired period, one drop of the mixed emulsion and dye was diluted with five cubic centimeters of sterile salt solution and injected subcutaneously into guinea-pigs. Table 7 summarizes both growth and animal experiments as to the power of the various dyes to kill the tubercle bacillus. In all the dyes except trypan blue, only one animal has been used, though the *in vitro* experiments have been repeated several times. In the one animal experiment, however, the time of exposure was 24 hours, and the other conditions were so severe that it seems reasonable to conclude that a given dye has little, if any, bactericidal power in case tuberculosis developed after the inoculation. In those cases in which no tubercle developed, however, no final conclusion will be drawn, but the experiment is being repeated on a larger number of animals. From this single experiment we can simply say that after 55 days' observation, no tubercle had developed in the animals inoculated with tubercle bacilli exposed for 24 hours to one per cent solutions of Ehrlich's rectified methylene blue, medicinally pure methylene blue, brilliant cresyl blue, and bismarck brown, while with gentian violet and neutral red, the development of a tubercle was much delayed none being found until after 35 and 47 days respectively. All the others developed local tubercles within 15 to 25 days.

SUMMARY.

1. Among the dyes so far tested, trypan blue, trypan red, isamine blue, pyrrhol blue, Ehrlich's rectified methylene blue, medicinally pure methylene blue, methylene blue of the U.S. Pharmacopeia, new methylene blue N, new methylene blue GG, and to some extent neutral red and pyronin have been found to penetrate tubercles in guinea-pigs. Basic fuchsin, crystal violet, and the other new methylene blues are now being tested, and a report will follow later.
2. The dyes above mentioned are well borne for a long period, if the dose of the methylene blues, basic fuchsin, and crystal violet is not too large. Almost any dose of the first four dyes mentioned is well borne.
3. The individual bacillus itself is penetrated and well stained by all the methylene blues, by basic fuchsin, and crystal violet, by erythrosin and the eosins; not so well by trypan blue, trypan red, isamine blue, pyrrhol blue, pyronin, and neutral red.
4. Methylene blue, bismarck brown, and brilliant cresyl blue are the only dyes which have a possible bactericidal power over the organism, though many of the others seem to inhibit its growth in the test tube.

Such are the conclusions from the work actually done. Practically the only work published on the therapeutic value of dyes in tuberculosis is the work of Gräfin von Linden and her coworkers on the so-called "Finkler Heilverfahren" with methylene blue. The therapeutic use of methylene blue is by no means new, since Ehrlich states that it was recommended to him as an internal antiseptic by Leopold Landau in 1880, and later Ehrlich found it *nicht ganz erfolglos* in neuralgia, while still later the same worker found it to have some specific influence on some cases of malaria. Its use in rheumatism, gonorrhea, and other infections is too well known to need mention. Gräfin von Linden claims that of the infected guinea-pigs treated with methylene blue chlorid or iodid, the local tubercles are healed in one-half to two-thirds of the animals, the temperature and weight and general condition are favorably influenced, and life is prolonged, in most cases the late death being due to some intercurrent infection. She also finds the dye penetrating the tubercle and staining the bacilli within the tubercle. Her clinical coworkers, Strauss and Meissen, also report some favorable results. While we have every reason to hope that methylene blue or some modification of that dye may have a favorable influence on tubercle bacillus infections, doubt is of necessity cast upon v. Linden's work, (1) on account of the small number of experimental animals treated with methylene blue, and (2) because Hugo Selter, originally one of her coworkers, states that the results are by no means conclusive.

My experiments with methylene blue have verified v. Linden's, so far as staining the tubercle bacilli *in vitro* and also penetrating the tubercle *in vivo*. I have, however, never found stained tubercle bacilli in the tubercle or in tuberculous pus stained with the dye.

I have for some weeks been treating tuberculous guinea-pigs with various preparations of methylene blue, but it is too early to make any report upon these, or even to judge as to whether any marked favorable influence is being exercised. I am also using therapeutically a number of other dyes and some modifications of trypan blue and allied dyes, which, it is hoped, will retain the favorable properties of trypan blue and at the same time have more marked bactericidal and curative properties. A report of these experiments will be published later.

It gives me great pleasure to acknowledge my indebtedness to Dr. H. Gideon Wells both for suggesting the work and for his constant and helpful interest in its progress.